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Title: ***USE OF CXCR4 AND ASTROCYTIC PROGENITOR RELATED PROTEIN EXPRESSION ON THE SURFACE OF NEURAL PRECURSORS AS A MARKER FOR TUMOR TROPIC POTENTIAL***

Atty. Dkt. 305907 **M#** | 81476 **Client Ref**

Date: October 6, 2003

including:
1. Specification: 16 pages   1A. ☒ Claim: 1 pages   1B ☒ 1 Abstract pages
2. ☐ Specification in non-English language   3. ☒ Drawings: 2 sheet(s)

4. The invention ☒ was ☐ was not made by, or under a contract with, an agency of the U.S. Government.
   If yes, Government agency/contact # = National Institutes of Health Grant No. NS02232

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(1) Inventor	John	S	YU
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

John S. YU and Moneeb EHTESHAM

Appln. No.: Unassigned

Filed: October 6, 2003

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Title: ***USE OF CXCR4 AND ASTROCYTIC PROGENITOR RELATED PROTEIN EXPRESSION ON THE SURFACE OF NEURAL PRECURSORS AS A MARKER FOR TUMOR TROPIC POTENTIAL***

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APPLICATION FOR A
PROVISIONAL UNITED STATES PATENT
IN THE NAME OF

JOHN S. YU AND MONEEB EHTESHAM

for

USE OF CXCR4 AND ASTROCYTIC PROGENITOR RELATED PROTEIN EXPRESSION ON THE SURFACE OF NEURAL PRECURSORS AS A MARKER FOR TUMOR TROPIC POTENTIAL

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USE OF CXCR4 AND ASTROCYTIC PROGENITOR RELATED PROTEIN EXPRESSION ON THE SURFACE OF NEURAL PRECURSORS AS A MARKER FOR TUMOR TROPIC POTENTIAL

The invention described herein arose in the course of or under Grant No. NS02232 between the National Institutes of Health and Dr. John S. Yu of Cedars-Sinai Medical Center.

Despite advances in surgical techniques and adjuvant therapies, the prognosis for patients with malignant glial tumors remains dismal. The median survival following diagnosis of glioblastoma multiforme, the most common and aggressive sub-type of malignant glioma, is under 1 year, with a 2-year survival rate approaching zero (1). The failure of currently employed therapeutic approaches, which center on surgical resection followed by radio- and/or chemotherapy, is rooted in the highly disseminated nature of these tumors. High grade gliomas are highly infiltrative neoplasms, with solitary tumor cells or clusters of neoplastic cells migrating throughout the brain, often to significant distance from the main tumor. Despite aggressive therapy, it is almost impossible to successfully eliminate all of these tumor foci which eventually serve as reservoirs for near universal tumor recurrence, thereby contributing to the inevitable lethality of this disease. Standard adjuvant treatments including radiation and chemotherapy have, despite having modest effects on long-term survival, been unable to effect any meaningful impact on patient prognosis. The development of a successful therapeutic modality for malignant glioma will, therefore, center on the ability to devise a means of eliminating all viable intracranial neoplastic reservoirs left behind after surgical resection of the primary tumor mass. At present, this remains a daunting task given the highly disseminated nature of the disease process, and our current inability to adequately visualize and therapeutically target every remaining tumor cell.

One promising means of specifically directing treatment to migrating tumor satellites that has recently come to light, involves the use of neural stem cells (NSC). NSC are multipotent progenitor cells that can be derived from either embryonic, fetal, neonatal, or adult tissues and are capable of long-term, sustained in vitro propagation and

terminal differentiation into neurons and glia. We and others have demonstrated that NSC exhibit potent tropism for disseminating glioma cells in vivo. When inoculated into established intracranial gliomas in rodents, NSC migrate away from the primary site of injection and intersperse themselves with, or track into proximity of, tumor satellites that have spread away from the primary tumor mass (2, 3). NSC engineered to secrete tumor toxic chemokines can, in this manner, deliver these therapeutic proteins directly to these disseminated neoplastic foci with significant bioactivity. In particular NSC populations secreting the immunostimulatory cytokines IL-12 and IL-4 as well as the pro-apoptotic protein TRAIL have been used to target migrating tumor pockets with resulting decreases in tumor burden and prolongation in survival (3-5). These findings indicate that the use of NSC represents a potentially viable tool for specific targeting of microscopic tumor nests that are otherwise refractory to currently employed therapies. However, despite promising results in pre-clinical murine models, the exact mechanisms governing the glioma tropic behavior of NSC are poorly understood. Additionally, our earlier observations demonstrated that while many intratumorally inoculated NSC demonstrated robust migratory activity and tumor tracking capabilities, a significant proportion of transplanted NSC did not exhibit this behavior and remained localized to the site of initial intracranial injection (3). This could be secondary to differing phenotypic profiles within our in vivo inoculated NSC populations, which comprise of progenitor cells at various levels of differentiation ranging from uncommitted multipotent precursors to cells that have initiated pathways leading to assumption of either a neuronal or glial fate (6). In this context, we hypothesized that the tumor tropic capacity we observed within our NSC inoculae was likely exhibited by a specific sub-population of progenitor cells at a particular stage of differentiation. We also wished to investigate whether in vivo glioma tracking NSC expressed any phenotypic markers such as chemokine receptors that would indicate responsiveness to known chemotactic cues related to NSC migration within the developing brain.

With the aim of further characterizing the tumor tropic component of the primary NSC populations utilized in earlier therapeutic models of intracranial glioma, we now describe that tumor tracking NSC comprise largely of astrocytic precursors expressing significant levels of CXCR4, a chemokine receptor that governs cellular migration and

homing in a variety of cell types including neuronal and glial precursors in the developing brain (7, 8). It has recently been reported that the production by glioma cells of stromal-cell derived factor-1 (SDF-1), the only known ligand for CXCR4, correlated with histological grade, tumor cell survival and invasiveness (9, 10). Based on the established roles of SDF-1 and CXCR4 in governing neuronal and glial precursor migration in the developing CNS, and the ability of invasive glioma cells to secrete SDF-1, we hypothesized that elaboration by disseminating tumor cells of this chemokine may play an important role in chemoattracting migratory NSC populations. We now demonstrate that the tropism of NSC towards glioma conditioned media in vitro can be inhibited by blocking cell surface CXCR4 receptors on NSC, further confirming the relevance of this pathway in NSC migration. These findings delineate important characteristics of the specific cells within generalized NSC populations that exhibit the therapeutically relevant behavior of "seek and destroy" tumor tropic migration. The use of these markers and further work on the characterization of these migratory sub-populations will allow for refining of NSC sub-populations that are increasingly responsive to cues that govern tropism for disseminated tumor satellites in vivo, and therefore allow for optimization of the therapeutic potential of NSC in this setting.

MATERIALS AND METHODS

Cells and culture process. The human U87MG and murine GL26 glioma cell lines were cultured in DM/F12 and DMEM (Invitrogen; Carlsbad, CA), respectively supplemented with 10% fetal bovine serum (Gemini Bio-Products; Calabassas, CA), L-glutamine and 1% Penicillin/Streptomycin (Invitrogen). Conditioned media from U87MG and GL26 cultures was obtained from confluent 75 cm2 culture flasks seeded 96 hours earlier with approximately similar numbers of cells. Cryopreserved human fetal NSC were obtained from Cambrex (Walkersville, MD) and murine NSC were harvested from the frontoparietal regions of day 15 mouse fetuses as described earlier (3). NSC were cultured in DM/F12 media (Invitrogen) supplemented with B-27 growth factor (Invitrogen), 1% Penicillin/Streptomycin (Invitrogen), and 2μg/ml heparin (Sigma; St. Louis, MO). Murine NSC were engineered to express β-galactosidase by means of in

vitro infection with LacZ gene bearing replication defective adenovirus as described previously (3).

Establishment of in vivo glioma model and NSC inoculation. Six to eight week old C57Bl/6 mice obtained from Charles River Laboratories (Wilmington, MA), were anesthetized with intraperitoneal ketamine and xylazine and stereotactically inoculated with 5x104 GL26 cells in 3μl of 1.2% methylcellulose/MEM in the right corpus striatum as reported previously (11). At day 7 postimplantation, animals received intratumoral inoculations of 2x105 β-galactosidase expressing NSC (NSC-LacZ) in 5 μl of serum and virus free media, injected directly into established tumor using the same burr hole and stereotactic coordinates. All animal use was performed in strict accordance with Institutional Animal Care and Use Committee guidelines in force at Cedars-Sinai Medical Center.

Immunohistochemical analysis of glioma tropic NSC phenotypes. Brains harvested from NSC-LacZ inoculated tumor bearing animals were frozen on dry ice, sectioned using a cryostat, mounted on slides, and then fixed in acetone. Staining was performed as per standard immunohistochemistry protocols using primary antibodies against β-galactosidase, Sox-2, SSEA-1, A2B5, E-NCAM, β-III Tubulin, glial fibrillary acidic protein (GFAP), CNPase, PDGFRα (Chemicon; Temecula, CA), CXCR4 (Torrey Pines Biolabs; San Diego, CA), EAAT1 and EAAT2 (Santa Cruz Biotech; Santa Cruz, CA). Secondary staining was performed using antibodies conjugated with the fluorophores FITC or Cy3 (Chemicon, Temecula, CA). Following staining, slides were mounted in aqueous mounting media (ICN Biochemicals; St. Louis, MO) and visualized under a fluorescence microscope.

In vitro chemotaxis experiments. All chemotaxis experiments were performed using a chemotaxis chamber system (Neuro Probe; Gaithersburg, MD) consisting of pairs of culture wells separated by a 5μm porous polycarbonate membrane. Lower wells were filled with either GL26 or U87MG conditioned media harvested as described above. Fresh DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin was used as a control (Unconditioned media). Following placement of the intervening porous membrane, approximately 1.5x105 disaggregated human or murine NSC were added to the top chambers. The chamber system was incubated at 37°C for 4 hours after which

media from lower wells was collected and quantitatively analyzed for cell content using flow cytometry against a defined number of fluorescent beads (BD Pharmingen; San Diego, CA). This allowed for quantification of the percentage of cells added to each top chamber that had migrated to the bottom chamber. For neutralization assays, anti-SDF-1 (250μg/l) (neutralizing both known α and β isoforms of the chemokine) and anti-CXCR4 (40μg/ml) monoclonal antibodies (R&D Systems; Minneapolis, MN) were incubated with tumor conditioned media or NSC, respectively, for 30 minutes at room temperature prior to the assay. Control samples were incubated with identical concentrations of an isotype matched non-specific antibody (BD Pharmingen; San Diego, CA). All experiments were performed in triplicate.

RESULTS

NSC that migrate to sites of disseminating tumor comprise of astrocytic precursors. We wished to determine whether the tumor tropism exhibited by inoculated primary NSC for in vivo disseminating glioma was localized to a particular sub-population at a specific stage of differentiation. We histochemically analyzed brain tissue from glioma bearing animals that had received intratumoral inoculations of NSC-LacZ. Routine X-gal staining revealed a significant proportion of β-galactosidase positive cells that had migrated away from the site of inoculation into proximity of islets of tumor cells (readily identifiable following a neutral red counterstain) that were disseminating into and through normal brain parenchyma (not shown), similar to findings reported by us previously (3). At the same time, a residual population of NSC-LacZ remained localized to the site of initial inoculation and did not exhibit this migratory, tumor tropic activity. We then subjected mirror sections of the above mentioned samples (i.e. analogous histological samples that were not more than 20-30 μm removed from the original samples visualized with X-gal staining), to immunofluorescent histochemistry with a panel of antibodies specific for markers reflective of proteins expressed at varying stages of NSC differentiation. These included the transcription factor Sox-2 and the cell surface antigen SSEA-1, known to be expressed in uncommitted neural precursors; A2B5 and E-NCAM, indicative of NSC that have initiated differentiation pathways towards astrocytic and neuronal fates, respectively; GFAP; expressed in cells of astroglial lineages; EAAT1

and EAAT2, glutamate transporter related proteins found in functional, differentiated astroglial cells; PDGFRα expressed in oligodendroglial precursors; CNPase, found in differentiated oligodendrocytes; and β-III tubulin, expressed in precursor as well as differentiated neuronal cells (6, 12-15). We specifically focused on expression of these markers in β-galactosidase positive NSC that had dispersed from the primary inoculation tract and were now migrating in conjunction with or in proximity to disseminating tumor satellites, as observed on earlier X-gal stained mirror sections. Our findings (summarized in Table 1) indicated that while populations of NSC expressing Sox-2 and SSEA-1 existed in the vicinity of the initial injection tract, the majority of β-galactosidase expressing NSC that were seen migrating along with glioma outgrowths and satellites were negative for these markers (not shown).

TABLE 1

Differentiation stage related marker	Differentiation stage	Staining on non-migratory NSC-LacZ	Staining on glioma tropic NSC-LacZ
Sox-2	multipotent NSC	weak, scattered cells	negative
SSEA-1	multipotent NSC	weak, scattered cells	negative
A2B5	glial restricted precursor, astrocyte restricted precursor, astrocyte	positive	positive
E-NCAM	neuronal precursor, neuron	weak, scattered cells	negative
PDGFRα	oligodenroglial precursor, oligodendrocyte	negative	negative
GFAP	astroglial precursor, astrocyte	strongly positive	strongly positive
β-III Tubulin	neuron	weak, scattered cells	negative
CNPase	oligodendrocyte	weak, scattered cells	negative
EAAT1 / EAAT2	differentiated glia (primarily astrocytes)	positive	negative

Additionally, these tumor tropic NSC populations were strongly positive for A2B5 and GFAP (Figure 1), while negative for the oligodendroglial associated proteins PDGFRα and CNPase (not shown) as well as the neuronal marker β-III tubulin (not shown), clearly indicating differentiation towards astrocytic lineages. At the same time, these cells were negative for the glial specific glutamate transporter related proteins EAAT1 and EAAT2, known to be expressed in differentiated astrocytes (13). Conversely populations of β-galactosidase positive cells with differentiated morphologies that expressed EAAT1 and EAAT2 along with GFAP and A2B5 could be observed in the vicinity of the initial injection tract within the main tumor mass (not shown), confirming

that complete astrocytic differentiation of inoculated precursors was, in fact, taking place. However, the absence of EAAT1/EAAT2 expression in glioma tracking β-galactosidase positive cell populations, in conjunction with expression of A2B5 and clear absence of fully differentiated morphology, indicate that tumor tropic cell populations likely comprised of progenitor cells that had initiated, but not completed, pathways towards astrocytic differentiation.

Tumor tracking NSC strongly express CXCR4. Based on the demonstrated ability of SDF-1 secretion from invasive glioma cells in promoting tumor invasiveness and survival (10, 16), as well as the established role of this chemokine and its receptor CXCR4, in governing neuronal and glial precursor migration within the developing brain (7, 8), we wished to investigate whether tumor tracking NSC-LacZ populations expressed CXCR4. We demonstrated that, while weak CXCR4 expression was visible both on glioma cells as well as within NSC-LacZ populations remaining within the main tumor mass (not shown), NSC-LacZ that were tracking tumor outgrowths and satellites strongly expressed this protein (Figure 1), indicating a potential role for this receptor in governing NSC responsiveness to glioma elaborated chemotactic cues.

NSC migration towards tumor conditioned media in vitro can be inhibited by blocking NSC surface CXCR4 receptors. Based on our observation that tumor tropic NSC populations in vivo strongly expressed CXCR4, we wished to determine whether this receptor played a role in NSC chemotaxis towards glioma. In a two-chamber based experimental system wherein tumor conditioned media was separated from human and murine NSC by a porous membrane, we observed that NSC migration towards glioma supernatant was significantly higher than that observed towards normal media (Figure 2), indicating chemotaxis towards a soluble factor present in tumor conditioned media. With the aim of determining whether neutralization of SDF-1 in tumor supernatant would inhibit NSC migration towards glioma conditioned media, we incubated an anti-SDF-1 antibody with human U87MG glioma tumor supernatant and then utilized this in a chemotaxis assay with human fetal NSC. We found that in comparison to the significant NSC chemotaxis seen towards U87MG supernatant incubated with a non-specific IgG isotype antibody, addition of the anti-SDF-1 neutralization antibody markedly decreased NSC migration (Figure 2A), although this difference did not meet statistical significance

(P=0.09; t-test). However, following incubation with an anti-CXCR4 blocking antibody, a significant decrease in NSC migration towards glioma conditioned media was seen both in the case of murine (Figure 2B) as well as human (not shown) fetal NSC (P=0.022 and P=0.003, respectively; t-test). In contrast, NSC incubated with an isotype matched non-specific antibody did not exhibit decreased migration towards tumor conditioned media when compared to untreated NSC (Figure 2B). These data indicate that blocking of CXCR4 significantly inhibits NSC taxis towards glioma supernatant, suggesting an important role for this receptor in the tumor tropic behavior exhibited by these cells. Our inability, however, to observe a statistically verifiable difference following neutralization of SDF-1 in tumor supernatants, may indicate either suboptimal neutralization of soluble chemokine or presence within the tumor conditioned media of secondary ligands capable of inducing chemotaxis through the CXCR4 pathway.

DISCUSSION

In the setting of malignant glioma, the use of tumor tropic NSC represents a promising approach capable of delivering tumoricidal therapeutic agents directly to disconnected neoplastic foci. The ability of NSC to track to isolated sites of disseminated tumor is fundamental to the success of this approach, and defining the mechanisms governing this behavior will prove critical to refining this therapeutic option for potential clinical translation. Recently it has been reported that high grade gliomas secrete significant levels of SDF-1, and that the expression of this protein and the CXCR4 receptor correlated with the histological grade and invasive capacity of these tumors, as well as tumor cell survival (9, 10, 16). Additionally, the interaction of CXCR4 and SDF-1 is a known factor involved in the migration of NSC (7), including astrocytic precursors, in the external granular layer of the developing cerebellum (8). We now demonstrate that glioma tracking populations of NSC within intratumorally injected primary NSC inoculae comprise of cells with phenotypic expression profiles characteristic of astrocytic precursors, as well as strong expression of CXCR4, and that the tumor tropic capacity of these cells can be inhibited by neutralization of surface CXCR4. These findings clearly point to astrocytic progenitors as the candidate cells exhibiting tumor tracking capacity within transplanted NSC, which is in keeping with earlier findings that immature astrocytes have the potential to migrate towards pathology in the brain (17). In this

context, it is important to note however, that Rao has described absence of GFAP along with expression of A2B5 as characteristic of astrocyte restricted precursors within populations of NSC (12). Our results, in contrast, demonstrate expression of both A2B5 and GFAP within tumor tropic NSC along with an absence of differentiated morphology and negligible expression of the glutamate transporters EAAT1/EAAT2, which are associated with functionally differentiated glial progeny. This may indicate that tumor tropic NSC exhibit an astrocytic precursor phenotype at a more advanced stage of differentiation than that reported by Rao. Additionally, of interest was our observation that expression of the neuronal markers E-NCAM and β-III tubulin was found in a very small proportion of transplanted NSC-LacZ. Although the corpus striatum has been shown to have a predominantly gliogenic influence on transplanted neural precursors (18), the paucity of neuronal differentiation we noted may potentially indicate the presence of factors inhibiting neurogenesis within the tumor microenvironment.

Based on the known chemotactic involvement of CXCR4 in neuronal and glial precursor migration in the developing CNS (7, 8), we wished to investigate whether this receptor played a functional role in NSC tropism towards tumor. Our observation that blocking of CXCR4 inhibits NSC tropism for glioma in vitro fundamentally supports the involvement of this receptor in mediating this behavior. It is important to note, however, that in spite of demonstrating marked reductions in mean NSC translocation following neutralization of SDF-1, we were, despite repeated experimentation, unable to validate these differences statistically. This may represent a technical issue involving suboptimal neutralization of soluble chemokine versus more efficient blocking of cell surface CXCR4, or these findings may point to a role for additional, as of yet unidentified soluble ligand(s) for CXCR4, possibly further isoform variants of SDF-1 apart from the α and β subtypes we neutralized. Nevertheless, as SDF-1/CXCR4 mediated astrocytic precursor dissemination may play a key role in the establishment of a glial scaffold in the developing brain, and the elaboration of SDF-1 by disseminating glioma cells is implicated as a key factor in their invasive properties, it is likely that similar mechanisms govern the tropism of NSC for migrating glioma cells.

The level of NSC migration we observed towards glioma conditioned media in vitro was significantly lower than that qualitatively predictable based on our previously

described in vivo migration patterns (3). This is, however, in conjunction with our finding that tumor tropic behavior is exhibited principally by cells that are progressing towards astrocytic differentiation. As the cells utilized in our in vitro experiments comprised chiefly of NSC cultured in conditions designed to favor maintenance of an undifferentiated state, although early evidence of eventual neuronal or glial directionality may still be discernable (12), a lower percentage of committed and actively differentiating astrocytic precursors would be expected in these populations. Following in vivo transplantation, however, NSC respond to predominantly gliogenic cues inherently present in the corpus striatum, increasing the numbers of astrocytic progenitors potentially responsive to chemotactic signals emanating from disseminating tumor cells. This is further supported by our observation that NSC populations maintained in culture contain numerous cells positive for the uncommitted precursor marker Sox-2 (M. Ehtesham; unpublished results) whereas expression of this factor was very weak in transplanted NSC-LacZ, 1 week following in vivo inoculation (Table 1). These findings may support a rationale for intratumoral transplantation of partially differentiated CXCR4 positive astrocytic progenitors rather than generalized pools of NSC. In particular, engineering A2B5/GFAP positive astrocytic precursors to overexpress CXCR4 may prove an attractive strategy to promote glioma tropism and improve the therapeutic potential of these cells. However, given that cerebral white matter, the most relevant site for inoculation of tumor therapeutic NSC, is known to already provide an environment favoring differentiation of NSC into astrocytes, it is unclear whether introduction of partially differentiated cells would provide any tangible benefit as opposed to the use of uncommitted NSC.

Also of interest was our finding that primary murine fetal NSC exhibited significantly more migration, even towards unconditioned media, as opposed to human fetal NSC. This may be explained by the differing origins of these cultures. Murine NSC were derived from primary fetal tissue whereas human fetal NSC were cultured from a several year old cryopreserved, commercially available stock. It is possible that freshly generated primary murine cells displayed a more active migratory capacity as opposed to the human NSC, whose biological activity may have been hampered secondary to prolonged cryogenic storage.

Given the abysmal prognoses associated with high grade gliomas, there is an urgent need to develop novel therapies with translational potential. Currently, the use of NSC as therapeutic delivery vehicles has offered encouraging results in pre-clinical models. The use of this technology in patients is still however hampered by significant limitations, key among which is the isolation of clinically viable and legally utilizable sources of tumor tropic neural progenitors. Progress is, however, being made on this front as exemplified by recent reports by our group and others regarding alternative tissue sources from which multipotent neural precursors can be derived (19, 20). Additionally, preliminary evidence indicates that these cells may also be tactic for migrating glioma cells (P. Kabos; unpublished results). The identification of CXCR4 as a key element governing the process of neural precursor migration towards glioma cells may allow for more efficient isolation of potentially tumor tropic cells from these alternative tissues, thereby hastening the therapeutic testing of glioma tracking neural precursors in a clinical setting.

FIGURE AND TABLE LEGENDS

Table 1. Expression of protein markers associated with differentiation of NSC on *in vivo* intratumorally inoculated NSC-LacZ.

Figure 1. NSC tracking tumor outgrowths and satellites *in vivo* are likely astrocytic precursors and express CXCR4. β-galactosidase expressing NSCs tracking disseminated glioma on histological sections of treated tumor bearing brains, were identified and sections stained using fluorescent histochemistry for a variety of neuronal and glial differentiation specific markers. NSCs within tumor outgrowths and satellites were positive for the astrocytic precursor marker A2B5 and GFAP (top and center rows, respectively) and negative for the neuronal commitment and differentiation markers E-NCAM and β-III tubulin (not shown). Additionally, tumor tracking NSC strongly expressed CXCR4, the cell surface receptor for SDF-1.

Figure 2. NSC demonstrate migratory tropism towards glioma conditioned media *in vitro*. Human and murine fetal NSC were placed in the upper well of a two-well chemotaxis chamber system, separated from a lower well containing various media/culture supernatants by a polycarbonate membrane with multiple 5 micron pores.

Following incubation at 37 degrees for 4 hours, media from the lower chambers was harvested and cells quantified. Y-axis depicts percentage of NSC that migrated into the lower chambers. ***A.*** Results indicated that human fetal NSC demonstrated minimal migratory activity towards normal unconditioned medium, whereas movement towards U87MG glioma supernatant was significantly higher (***P***=0.005; t-test). Dilution of glioma media resulted in a significant decrease in NSC chemotaxis (not shown) indicating that NSC translocation was likely due to a tumor elaborated soluble factor. Addition of a neutralizing antibody against one such potential factor, SDF-1, reduced chemotaxis noticeably compared to NSC treated with isotype IgG, albeit not to a statistically significant extent (***P***=0.09; t-test). ***B.*** Murine fetal NSC demonstrated enhanced migratory activity towards GL26 conditioned medium compared to control media (***P***=0.0001; t-test). Addition of an anti-CXCR4 neutralization antibody significantly decreased NSC translocation towards glioma conditioned media compared to NSC treated with isotype IgG (***P***=0.003; t-test).

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While the description above refers to particular embodiments of the present invention, it will be understood that many modifications may be made without departing from the spirit thereof. The accompanying claims are intended to cover such modifications as would fall within the true scope and spirit of the present invention. The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims, rather than the foregoing description, and all changes that come within the

meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A method for assessing tumor tropic potential of a neural precursor cell, comprising:

providing a neural precursor cell;

determining an expression level of CXCR4 by the neural precursor cell; and

assessing tumor tropic potential of the neural precursor cell based upon said expression level of CXCR4.

ABSTRACT

Malignant gliomas spawn disseminated microsatellites which are largely refractory to currently employed therapies, resulting in eventual tumor recurrence and death. The use of tumor tropic NSC as delivery vehicles for therapeutic gene products represents an attractive strategy specifically focused at treating these residual neoplastic foci. We wished to elucidate the biological cues governing NSC tropism for glioma. In this context, we describe that tumor tropic NSC comprise largely of astrocytic progenitors expressing CXCR4. Blocking of CXCR4 significantly inhibits NSC migration towards tumor. These findings define specific characteristics associated with the cell populations within transplanted NSC that demonstrate glioma tracking behavior.

FIGURE 1

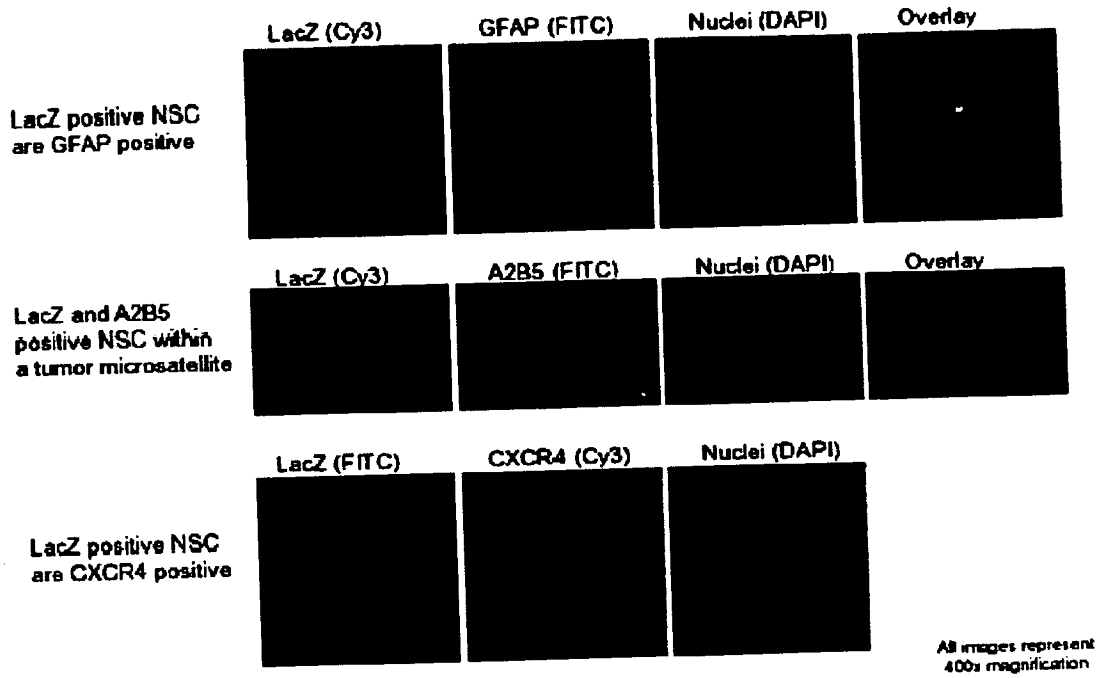
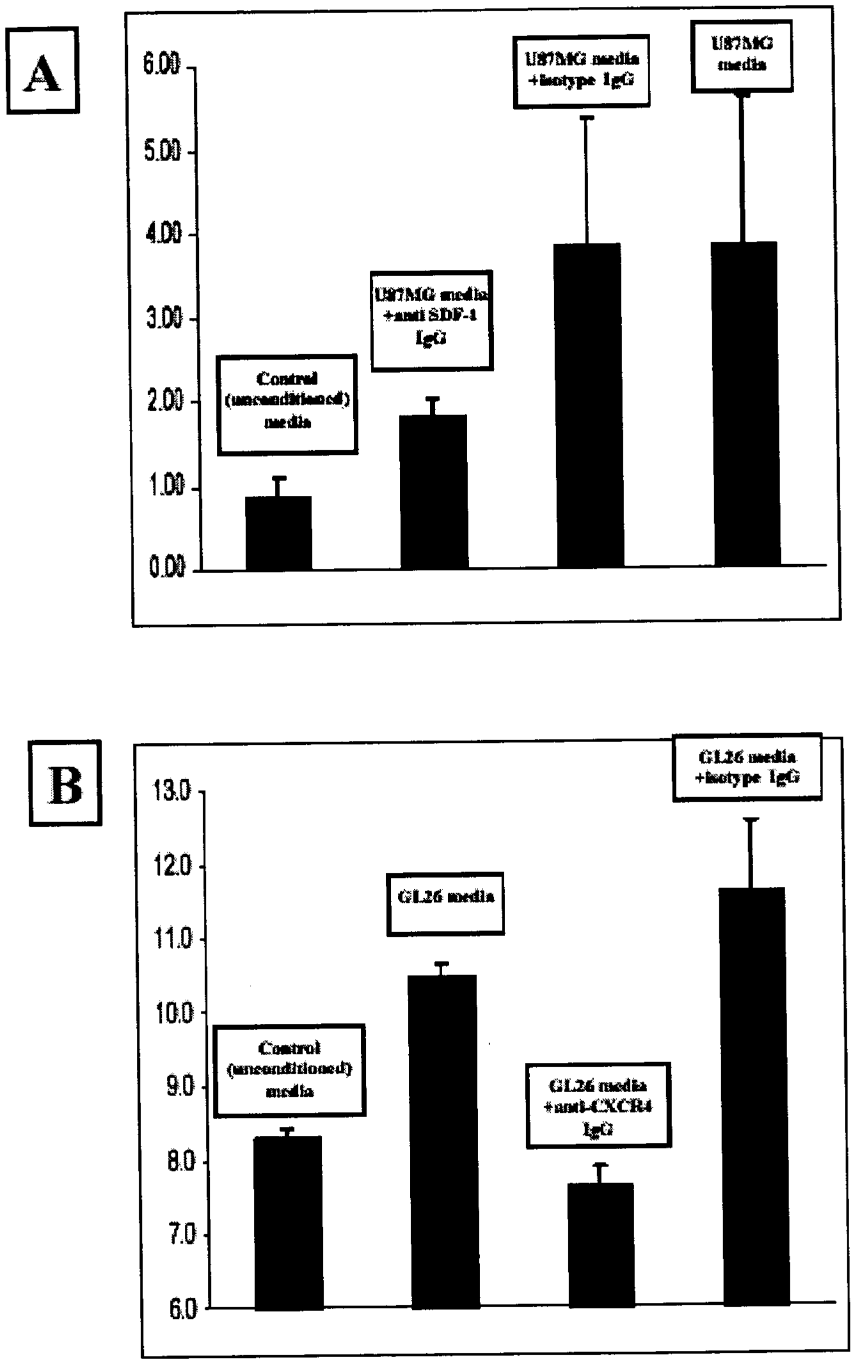

LacZ (Cy3)
GFAP (FITC)
Nuclei (DAPI)
Overlay
LacZ positive NSC are GFAP positive
LacZ (Cy3)
A2B5 (FITC)
Nuclei (DAPI)
Overlay
LacZ and A2B5 positive NSC within a tumor microsatellite
LacZ (FITC)
CXCR4 (Cy3)
Nuclei (DAPI)
LacZ positive NSC are CXCR4 positive
All images represent 400x magnification

FIGURE 2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Control (unconditioned) media
GL26 media
GL26 media +anti-CXCR4 IgG
GL26 media +isotype IgG